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Thermopreference Behavior of Bluegill (*Lepomis macrochirus*) Subjected to Restrictions in Available Temperature Range

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Juvenile bluegill (*Lepomis macrochirus*) acclimated to 25 C occupied a temperature range of 29.5 to 33.1 C in laboratory preference studies. The midpoint of this range, 31.2 C, is considered an estimate of the final temperature preferendum for this species. Experiments were designed to determine 1) the behavioral response of bluegill to situations where available temperatures were either (a) greater or (b) less than their preferendum; and 2) whether a constant temperature approximately either 1 C above or below the limits of the occupied range would influence thermoselection behavior by bluegill.

When the temperature preferendum was unavailable, bluegill spent more than 92% of their time at the temperature nearest the preferendum. The availability of constant non-preferred temperatures appeared to have little influence on thermoselection behavior: median percent time spent at the two non-preferred temperatures, 28.0 and 34.0 C, were 9.1% and 1.3%, respectively. In addition, a separate experiment demonstrated that of the two limits of the preferred range, the lower limit is most labile. These findings are related to the thermal ecology of the bluegill.

ALTHOUGH all known freshwater fishes are ectotherms, an abundance of field and particularly laboratory evidence exists indicating that fish are not strictly at the mercy of local ambient temperatures. As highly mobile animals, possessing acute temperature discrimination capabilities (Bull, 1936-37; Bardach and Bjorklund, 1957) fish are able to take advantage of the heterothermal quality of aquatic environments by behaviorally seeking out and occupying water temperatures favorable to successful functioning of their physiological and biochemical processes. When placed in laboratory temperature gradients, fish typically congregate within certain temperatures, while actively avoiding others. As behaviorally determined parameters, preferred and/or avoidance temperatures of many fish species are influenced by physical (light intensity, Sullivan and Fisher, 1954), chemical (pesticides, Anderson, 1971), and biological (social behavior, Beitinger and Magnuson, 1975) factors.

Research concerning the influence of temperature itself on temperature selection behavior of fishes can be divided into three general headings: 1) temperature acclimation (i.e., thermal history of subjects prior to tests), 2) thermally related "dosing" of subjects during

testing (i.e., manipulations causing changes in the temperature of subjects during testing) and 3) gradient variability (i.e., how temperature is presented to test subjects).

The relationships between thermoselection behavior and pretest exposure of subjects to constant (Doudoroff, 1938; Fry, 1947) and cyclic temperature regimes (Reynolds and Thomson, 1974) have been investigated. Examples of experimental "dosing" of fish during temperature preference testing include Crawshaw and Hammel's (1974) artificial brainstem heating and cooling experiments, temperature shock research (Beitinger, 1974) and induced "fever" experiments of Reynolds, Covert and Casterlin (1976). Variations in the direction (horizontal and vertical) of spatial temperature gradients (McCauley and Pond, 1971) and rate of temperature change in temporal gradients (Rozin, 1968; Beitinger, 1976) exemplify efforts demonstrating the influence of gradient variability on fish thermoselection behavior. The results of these experiments have increased our understanding of the capability, stability, precision and underlying physiology of fish thermoselection behavior.

Experiments reported herein were designed to examine the behavioral responses of fish to a

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different type of gradient variability, available temperature range. In a modified temporal temperature gradient, individual bluegill (*Lepomis macrochirus*) were exposed to 6 different potential temperature regimes to determine if the observed temperature preference and avoidance responses would follow those expected from preferred temperature theory.

MATERIALS AND METHODS

Specimens.—Juvenile bluegill (mean \pm standard deviation = 71.9 ± 10.1 mm SL) were captured by electroshocking during summer from Lake Wingra (Dane Co., Wisconsin). Fish were maintained in the laboratory at 25 C (exceptions noted) and constant LD (i.e., light/dark) 14:10 photoperiod for at least two weeks prior to examination. Before and during experiments, fish were fed trout pellets once daily.

Apparatus.—The test apparatus was described in detail by Beitinger (1974) and Beitinger et al. (1975). The design (Neill et al. 1972) substitutes a modified temporal gradient of temperature for the strictly spatial gradient typical of most gradient studies. Each 50-liter test tank was divided into halves by a fiberglass partition. A tunnel in the partition allowed the subject to choose between tank halves which always differed by a constant temperature, usually 2 C. Fish movement through the tunnel was monitored by a pair of photoelectric cells. Passage of the fish into the warmer half of the tank triggered the temperatures of both tank halves to increase at a constant rate (3.0 C/h), and when a fish swam into the cooler tank half, the temperature in the entire tank decreased at 3.0 C/h. This design allows fish to control its thermal exposure by simply moving from one side of the tank to the other.

Procedures.—One fish was introduced per tank and allowed to experience the system for 2.5 days with tank halves set at constant temperatures 1.0 C above and 1.0 C below the fish's acclimation temperature. Then the temperature rate-change mechanism was activated and tank temperature control was relinquished to the fish for 4 days. Thermoregulatory performance was assessed from data collected during the second, third and fourth days.

Temperatures of each tank half were monitored by a thermistor-wheatstone bridge circuit connected to a multichannel analog recorder. The following 4 interrelated parameters were utilized to describe the thermoregulatory

performance of test fish: 1) lower avoidance temperature; 2) upper avoidance temperature; 3) width of the preferred range; and 4) preferred temperature. The first two parameters are the same as the "turnaround" temperatures defined by Neill and Magnuson (1974). The width of the preferred range was computed as the positive arithmetic difference between the two avoidance temperatures. Finally, the preferred temperature was operationally defined as the temperature midway between the two avoidance temperatures.

Fish were randomly exposed to one of the following six different temperature regimes:

- A. Control situation. Data from 32 bluegill during the pretreatment portion of experiments reported by Beitinger (1974) were utilized to compute thermal preference and avoidance statistics. These fish were collected at the same time and held in the laboratory under the same conditions as bluegill utilized in thermal restriction experiments. During tests fish had available temperatures of 5 to 55 C, and hence were considered to be thermally unrestricted.
- B. Experimental conditions (5 in total).
 1. Fish were given access to temperatures of 5 to 55 C, except that a 4 C (instead of the usual 2 C) tank half temperature differential was employed. Since 4 C was greater than the mean preferred range of control fish, this manipulation forced fish to:
 - a. increase their upper avoidance,
 - b. decrease their lower avoidance, or
 - c. both,
 in order to regulate temperatures around their final preferendum. These experiments were conducted to determine which avoidance temperature was more labile.
 2. Available temperature ranges were restricted by the imposition of various maxima or minima. Whenever the temperature of a tank half reached a limit, the temperature rate change stopped, hence, temperatures of both sides remained constant (± 0.1 C) until the fish again swam into the alternate half of the tank. At all times the 2 C differential was maintained between tank halves. The percent time spent at the imposed limit was determined for each fish. Limits either included or excluded the preferred temperature of 31.2 C derived from control bluegill.

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TABLE 1. COMPARISON BETWEEN THERMOREGULATORY PERFORMANCE OF 9 BLUEGILL EXPOSED TO A 4 C TANK-HALF TEMPERATURE DIFFERENTIAL, AND 32 BLUEGILL EXPOSED TO THE USUAL 2 C DIFFERENTIAL. Values are means \pm standard deviations in C. The third row of numbers are probabilities from Mann-Whitney U comparisons between the 2 groups for each thermoregulatory parameter.

Thermoregulatory Parameter	Fish Under 2 C Differential	Fish Under 4 C Differential	Mann-Whitney Probability ^a
Lower Avoidance Temperature	29.3 \pm 0.8	27.9 \pm 1.1	0.001
Upper Avoidance Temperature	33.1 \pm 0.7	33.0 \pm 0.8	0.90
Preferred Temperature	31.2 \pm 0.7	30.5 \pm 0.9	0.02
Preferred Range Width	3.8 \pm 0.7	5.1 \pm 0.5	0.0001

^a Probabilities of ≤ 0.05 were considered significantly different.

- a. Preferred temperature (i.e., 31.2 C) unavailable.
 1. Maximum available temperature equalled 28 C. Fish had access to temperatures of 5 C to 28 C.
 2. Minimum available temperature equalled 32 C. Temperatures of 32 C to 55 C were available to test fish.
- b. Preferred temperature available.
 1. Minimum available temperature equalled 28 C. Fish had access to temperatures of 28 C to 55 C.
 2. Maximum available temperature equalled 34 C. Temperatures of 5 C to 34 C were available to test fish.

RESULTS

Control data.—Fish in unrestricted thermal environments had mean lower and upper avoidance temperatures of 29.3 and 33.1 C, a preferred temperature of 31.2 C, and mean preferred range width of 3.8 C.

Tank-half differential of 4.0 C.—Bluegill demonstrated no reluctance to traverse the 4 C temperature gradient between tank halves. Comparisons between thermoregulatory perfor-

mances of bluegill exposed to 4.0 C and 2.0 C differentials indicated that only the upper avoidance temperature was not significantly affected (Table 1). The obvious difference in width of preferred range was expected (Mann-Whitney U test, $P = 0.0001$). The 1.4 C decrease in mean lower avoidance temperature was significantly different at the $P = 0.001$ level, and was responsible for the 0.6 C decrease in mean preferred temperature observed in these fish.

Thermal restriction experiments.—When the preferred temperature was unavailable, fish spent the majority of their time at the temperature nearest to the preferred temperature. The 10 fish exposed to the 28.0 C maximum available temperature spent 92.5% of their time at this temperature, while 5 fish tested at the 32.0 C minimum available temperature spent 98.9% of their time at the minimum (Table 2). Time spent by individual bluegill at these two limits were significantly different (Mann-Whitney U, $P < .01$).

Fish operating under thermally restricted conditions when the preferred temperature was present spent relatively little time at the available constant limits (Table 2). Although 4 of

TABLE 2. SUMMARY OF RESULTS OF THERMAL RESTRICTION EXPERIMENTS. Time at the imposed thermal minima and maxima by fish of each experimental group are given.

Imposed Limit	Fish Tested	Number of Fish Experiencing Limit	Experimental Time (hr)	Percentage Time at the Thermal Limit	
				Median	Interquartile Range
1. Preferred Temperature Unavailable					
28 C maximum	10	10 (100%)	720.0	92.5	90.0 to 97.9
32 C minimum	5	5 (100%)	200.0	98.9	98.2 to 99.2
2. Preferred Temperature Available					
28 C minimum	10	6 (60%)	396.8	9.1	1.7 to 18.8
34 C maximum	16	12 (75%)	817.4	1.2	0.4 to 5.2

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the 10 fish exposed to the 28.0 C minimum limit did not decrease their cooler tank temperature to the limit, median time at the minimum was 9.1% for the remaining 6 fish. Four of the 16 fish tested with the 34.0 C maximum available temperature did not cause the warmer tank temperature to reach 34.0 C. Median time at the maximum temperature equalled 1.2% for the remaining 12 fish. Owing to the high variability, times spent by individual fish at these 2 limits were not significantly different (Mann-Whitney U, $P = 0.16$).

DISCUSSION

In thermally unrestricted experiments, bluegill maintained tank temperatures within 2 precise bounds, the lower and upper avoidance temperatures (Table 1). The calculated preferred temperature of 31.2 C is within 1 C of other estimates for bluegill (Fry and Pearson, pers. comm.; Neill and Magnuson, 1974; Beiting, 1975; Cherry et al., 1975; Reynolds and Casterlin, in press). These laboratory-determined preferred temperatures are similar to the estimated temperature acclimation state and deep muscle temperature of young bluegill collected during summer in an outfall zone of a power plant on Lake Monona, Wisconsin (Neill and Magnuson, 1974).

Because the observed preferred range of the bluegill is much closer to the incipient upper lethal temperature, ~36.5 C (Speakman and Krenkel, 1971) than to the incipient lower lethal temperature, I postulated that bluegill would allow less variation in their upper avoidance temperature than in their lower avoidance temperature. Results from fish exposed to 4.0 C tank-half differential support this hypothesis (Table 1). The mean lower avoidance temperature of these fish was significantly less (1.4 C) than that of control fish; whereas mean upper avoidance temperature differed by only 0.1 C from the mean control value. These data suggest that the upper avoidance temperature is regulated more stringently than the lower avoidance temperatures and probably represents the highest temperature at which bluegill will voluntarily reside. In his review of fish temperature preference data, Coutant (1975) concluded that the upper avoidance temperature is more sharply defined than the lower avoidance temperature for most species.

Few warm-water fish species live in natural environments where the annual temperature range exceeds their preferred temperature

range. However, the converse situation in which available water temperature seldom attains preferred levels or where the amount of water at preferred temperature is small relative to the total water volume is more usual for warm-water species in temperate climates. Behavioral responses of fish to these situations were examined in experiments where the preferred temperature was unavailable to test fish. The results overwhelmingly demonstrate that of the available temperatures, bluegill spent more than 90% of their time at temperatures nearest their preferendum (Table 2). Test fish spent significantly less time at the 28 C maximum limit than the 32 C minimum limit. This difference is explained by the observation that 32, but not 28 C, is within the preferred range of the bluegill. Also, 26 C, with decreasing temperatures, is less stressful than 34 C with increasing temperatures.

The selection of temperatures nearest to a species' preferendum has been observed in nature (Ferguson, 1958; Neill and Magnuson, 1974). Gammon (1973) reported that among the several thermally diverse areas available in the Wabash River during summer, most fish species selected the warmest temperature available, but only a relatively well defined "highest" (upper avoidance) temperature. Similar results were found by telemetry for largemouth bass (*Micropterus salmoides*) in a Tennessee quarry (Coutant, 1975). During March, tagged fish consistently occupied the warmest available temperature in shallow nearshore waters. However, when the surface waters began to exceed about 26 C in July, bass were observed to avoid warmer shoreline zones and remain in deeper waters at temperatures of about 25-29 C. These temperatures approximate the estimated laboratory preferred temperature for bass of this size (Reynolds et al., in press).

Seeking out warm waters represents a good strategy for most fishes. Warmer (sublethal) environments are more conducive to fish growth and locomotor activity and typically offer an energetic bonus in the form of increased food availability.

Because bluegill spent the most time at the constant thermal limits I wondered whether these temperatures were selected because they were constant or because of the available temperatures they were nearest to the preferred temperature. The next 2 experiments offered bluegills the opportunity to remain at constant temperatures (either 28.0 or 34.0 C) slightly outside of their preferred range, when the preferred

range was available. In effect, fish were offered a choice between a constant non-preferred temperature and an opportunity to actively thermoregulate within their preferred temperature range. In both experiments, fish spent only a minor proportion of their time at fixed 28 or 34 C temperatures. Clearly, fish spent the majority of their time within their preferred temperature range. To appreciate this result, it is important to distinguish between the temporal thermal gradient employed in this study, and the spatial gradient of classical preferred temperature research. In the latter, a fish actively selects a temperature at which it can (but often does not) remain passively (Reynolds and Thomson, 1974). Owing to the dynamic temperature nature of the temporal gradient system, a fish must actively thermoregulate at all times to remain within its preferred temperature range. Consequently, the demands of this system should make the opportunity to reside at a constant temperature appealing. Nevertheless, bluegill thermoregulatory behavior was not overridden by the opportunity to reside at constant temperatures.

Combined results from these experiments strongly support thermal primacy theory and point to four general conclusions concerning the behavioral responses of bluegill to temperature: 1) bluegill are attracted to warm water, and have a preferred temperature range of 29-33 C; 2) the upper avoidance temperature is apparently more critical as it is regulated more closely than the lower avoidance temperature; 3) if the preferred temperature is not available, bluegill select from the available range those temperatures closest to their preferred temperature; and 4) availability of constant temperatures just outside the preferred temperature range does not override the bluegill's thermoregulatory behavior. The results of these experiments point to the high degree of precision and stability of behavioral thermoregulation in this species.

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Cytogenetic Studies in North American Minnows (Cyprinidae)

I. Karyology of Nine California Genera

J. R. GOLD AND J. C. AVISE

Karyotypes of nine species representing nine genera of cyprinid fishes inhabiting California were examined. The nine genera, including *Hesperoleucus*, *Lavinia*, *Mylopharodon*, *Pogonichthys*, *Ptychocheilus*, *Orthodon*, *Richardsonius*, *Gila* and *Notemigonus*, all have diploid chromosome numbers of 50. *Notemigonus* is the only genus non-native to California, having been introduced from the eastern United States. Measurements of centromeric indices suggested differences in fundamental arm number among the genera. In addition, one long chromosome with a distally located centromere was observed in the karyotype of each species, and may be of future use in North American cyprinid systematics.

THE cyprinid fishes endemic to the North American continent include a vast array of different forms thought to belong (with a single possible exception) to one subfamily, the Leuciscinae (Miller, 1959). It is generally believed that most North American cyprinids are of relatively recent phyletic origin and may share a common ancestry stemming from a few cyprinid fishes which migrated from Eurasia during the Miocene (Miller, 1959, 1965).

Recently, Avise et al. (1975) and Avise and Ayala (1976) reported studies on biochemical-genetic differentiation among nine genera of

cyprinid fishes inhabiting California. Their results suggested that four genera were similar in genic content and were perhaps of monophyletic origin. The remaining five genera were less closely related genically, and may represent the mean levels of genic divergence between all North American cyprinids.

In the present study, we have examined the karyotypes of the nine taxa studied by Avise and Ayala (1976). Our results indicate that all nine genera (including one genus native to the eastern United States and possibly a member of a different subfamily, the Abramidinae) are